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Relationship Between the ATP (Adenosine Triphosphate) Content of Subsurface Material and the Rate of Biodegradation of Alkylbenzenes and Chlorobenzene

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RELATIONSHIP BETWEEN THE ATP CONTENT OF SUBSURFACE MATERIAL AND THE RATE OF BIODEGRADATION OF ALKYLBENZENES AND CHLOROBENZENE

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ABSTRACT

Wilson, J.T., Miller, G.D., Ghiorse, W.C. and Leach, F.R., 1986. Relationship between the ATP content of subsurface material and the rate of biodegradation of alkylbenzenes and chlorobenzene. In: D.L. Macalady (Editor), Transport and Transformations of Organic Contaminants. J. Contam. Hydrol., 1: 163—170.

The rate of biotransformation of toluene in unconsolidated subsurface material from sites at Lula, Oklahoma, and Conroe, Texas, was compared to the ATP (adenosine triphosphate) content of these materials. The rate of toluene degradation decreased with decreasing ATP content. When ATP contents were at or less than 0.05 ng g^{-1}, biotransformation of toluene could not be detected (less than 1% of the initial concentration was degraded per week). At intermediate concentrations of ATP, 0.37 and 0.16 ng g^{-1}, the rates of toluene degradation were 18 ± 4.5% and 25 ± 10% of the initial concentration per week. At ATP concentrations above 1 ng g^{-1}, the rates of toluene degradation exceeded 90% of the initial concentration per week. There was no simple relationship between ATP content and chlorobenzene degradation. Subsurface material that had been exposed to creosote wastes, and which degraded toluene rapidly, also degraded benzene, o-xylene, and m-xylene.

INTRODUCTION

Petroleum-derived hydrocarbons are common organic contaminants of groundwater. Toluene and other alkylbenzenes are among the more water-soluble components of gasoline and fuel oils and are released into groundwater from spills of these materials (Zurcher and Thuer, 1978). They are also commonly encountered in leachate from municipal landfills and hazardous waste disposal sites (Reinhard et al., 1984). Chlorobenzene is also commonly found in groundwater polluted by hazardous waste sites.

Estimates of the rate of biodegradation of these compounds in subsurface materials are needed to assess damage at existing contaminated sites, to

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regulate activities that may release these materials to the subsurface, and to select the best alternative to clean up existing sites (Dunlap et al., 1984). At present these estimates are only available through expensive, tedious, and lengthy microcosm studies (Wilson et al., 1983). There is a need for an index to predict biotransformation that can be determined rapidly and at low cost. The total number of cells as determined by direct examination is not a good index because the numbers are surprisingly uniform from one site to another and at different depths in the same site, even though biodegradation potential varies widely in the same material (Novak et al., 1984; also compare Webster et al., 1985, and this paper).

ATP (adenosine triphosphate) is an integral part of the energy metabolism for all terrestrial life. A sensitive assay is available for its determination in subsurface material. This study examined the usefulness of ATP concentration as an index for predicting the biodegradation of toluene, other alkylbenzenes, and chlorobenzene in subsurface material. The ATP content of subsurface material from two field sites has been reported previously (Webster et al., 1985). This paper compares the ATP content of the subsurface material reported in Webster et al. (1985) to the rate of biodegradation of toluene and other organic pollutants by microbes in the same subsurface material.

BIOTRANSFORMATION ASSAY

All work was done on samples that were acquired in a manner that prevented contamination from surface microorganisms (Wilson et al., 1983).

Core material was stirred with additions of sterile distilled water until it became a slurry. This slurry was aseptically transferred to 40-ml screw-cap test tubes; then 1.0 ml of a sterile aqueous solution of toluene and other organic compounds was added to each tube. Then the tubes were sealed with a Teflon-lined screw cap and the organic compounds mixed through the slurry with a vortex mixer. Final concentrations of the organic contaminants ranged from 100 to 600 $\mu g\,l^{-1}$. Samples were analyzed after 0, 1, 2, 4, 8, and 16 weeks. At each time period, three test-tubes were analyzed. The pore water was diluted and the concentrations of organic compounds were determined by the purge-and-trap method (see Wilson et al., 1983 for details). Recoveries exceeded 90%; the detection limit was 1 $\mu g\,l^{-1}$.

Precautions were taken to insure that the pore water of the slurries contained at least 0.5 $mg\,l^{-1}$ of oxygen. The microcosms were incubated in the dark at the temperature of collection (25° and 17°C for material from Conroe, Texas and Lula, Oklahoma, respectively). Portions of core material were autoclaved prior to some of the assays in order to distinguish biotransformation from abiotic processes.

PRISTINE SITE AT LULA, OKLAHOMA

The behavior of toluene and chlorobenzene in pristine subsurface material was studied in unconsolidated material from the margin of the flood plain of a small river near Lula, Oklahoma. As are many riverine deposits, the unconsolidated section was very complex. However, the following discrete layers are apparent (Fig. 1). First, a fine silty-clay subsoil extends to the permanent water table near 2.5 m. This material has strong structure with many cracks and root channels. Second, a layer of fine sand about 0.5 m thick lies just below the permanent water table. This material has no structure, is very plastic, and contains enough clay to make it relatively impermeable. Third, a mixture of coarse sands and gravels extends from 3.0 to 4.5 m, followed by a layer of uniform sands extending to 5.6 m. These sands and gravels are moderately permeable. Fourth, below the sand is a layer of gravels down to 7.2 m. These gravels are very permeable. The "bedrock" is a massive olive-green clay. Water produced from the lower gravels is hard and well-oxygenated (Table 1). Water below the fawn-colored sand at 3.0 m is under 0.5 m of artesian pressure.

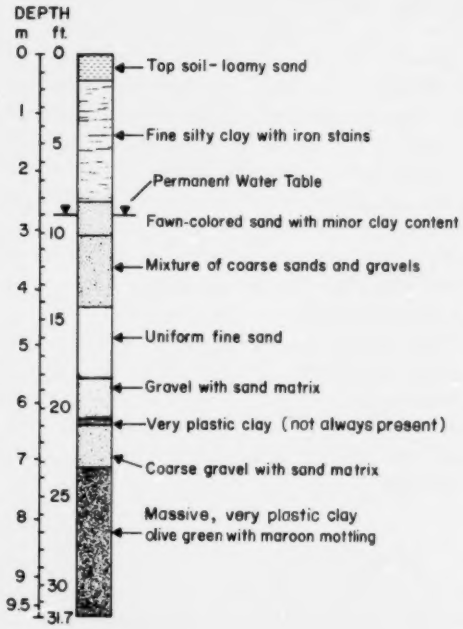

Fig. 1. Section of a pristine study site at Lula, Oklahoma.

TABLE 1

Chemistry of well water from study sites at Lula, Oklahoma, and Conroe, Texas

		Lula, Oklahoma	Conroe, Texas	
Parameter	Unit	Well No. 9V in pristine material	Well No. 7 in pristine material	Well No. 5B in material exposed to creosote wastes
temperature	°C	17	25	26
pH		7.1	4.0	4.7
conductivity	μmho cm^{-1}	1675	88	220
o-phosphate	mg l^{-1}	0.12	0.01	0.04
sulfate	mg l^{-1}	197	3	10
$NO_2^- + NO_3^-$—N	mg l^{-1}	0.8	0.05	1.2
NH_4^+—N	mg l^{-1}	< 0.05	0.01	0.1
chloride	mg l^{-1}	164	16	66
O_2	mg l^{-1}	2.6	1.4	0.2

CONTAMINATED SITE AT CONROE, TEXAS

The relationship between ATP content and alkylbenzene degradation in polluted subsurface material was studied at a site in Conroe, Texas. A disposal lagoon for wood-creosoting process wastes produced a plume of water containing a number of polynuclear aromatic hydrocarbons and polyheterocyclic hydrocarbons at individual concentrations of 800—200 μg l^{-1}, and toluene, benzene, m-xylene, o-xylene, and p-xylene at individual concentrations of 30—15 μg l^{-1}. The present water table is 7.6—8.5 m deep. The pollution is moving through a layer of permeable sand 2.0—2.5 m thick that lies above an impermeable layer of clay (see Fig. 2). Pristine water in the sand is acid, oxygenated, and low in salts (Table 1).

At the margin of the plume the contaminated water is mixed with oxygenated pristine water, which allows microbes in the aquifer to degrade the hydrocarbons and renovate the water in the plume. This renovated water can be recognized because it still contains elevated concentrations of chloride representative of the plume although the oxygen is depleted (Wilson et al., 1985; compare Wells No. 5B and 7 in Table 1).

RESULTS

At the Lula, Oklahoma site, the ATP content and rate of degradation of toluene varied widely over a relatively small depth interval (Table 2). In material from the unsaturated zone and material just below the water table,

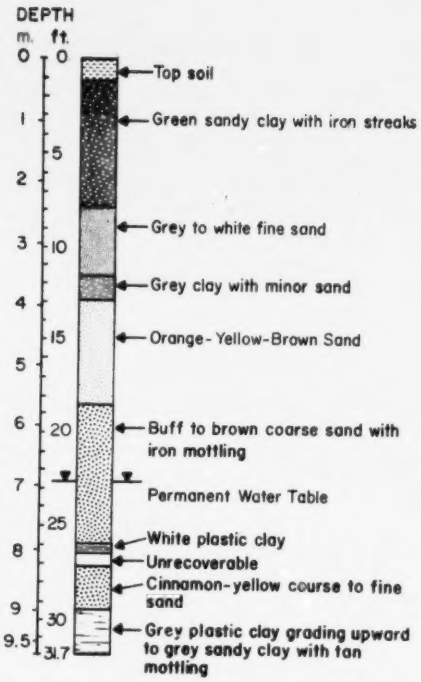

Fig. 2. Section of a study site at Conroe, Texas that received wood-creosoting wastes.

toluene degradation was very rapid. Considering the expected residence time of water at these depths, the rates are effectively instantaneous. These active subsurface materials had ATP contents near $1\,ng\,g^{-1}$. This is much lower than the ATP content of surface soils in Oklahoma, which is typically $44—500\,ng\,g^{-1}$ (Webster et al., 1985). The ATP content of material from regions deeper below the water table was much lower than that in the unsaturated zone. Toluene degradation was much slower at $0.14\,ng\,g^{-1}$ ATP and was undetectable at $0.03\,ng\,g^{-1}$. These deeper regions were under artesian pressure. The flow path from the point of recharge may have been much longer than is the case for material that receives water from directly above. This may have allowed time for organisms further up the flow path to remove more of the metabolizable components in the dissolved organic carbon.

The degradation of chlorobenzene was not detected in the material with the lowest ATP content. Chlorobenzene degradation was slow in the other materials and was not related in any simple way to the ATP content.

In pristine material from the site at Conroe, Texas, the ATP content was also low, less than $0.2\,ng\,g^{-1}$, and there was no detectable biodegradation

TABLE 2

Relationship between ATP content and biodegradation of substituted benzenes in subsurface material from a pristine site at Lula, Oklahoma

Core No.	Position	ATP content ($ng\,g^{-1}$)	Rate of disappearance % initial conc. per week	
			toluene	chlorobenzene
9E1	Fine silty clay with iron stains 1.5 m deep	1.2		
NA[b]	Same at 1.2 m		> 92	5.1 ± 4.6[a]
9D1A	Fawn-colored sand with minor clay content (not transmissive) 2.9 m deep	1.1		
9D1B	Same at 2.9 m	1.9		
NA	Same at 3.0 m		> 91	5.4 ± 3.3
9G1	Uniform fine sand 4.6 m deep	0.16		
9I1 + 9I2	Same at 5.5 m		25 ± 10	20 ± 13
9G3	Gravel with sand matrix 6.1 m deep	0.03		
9Q7 + 9Q8	Same at 6.7 m		− 0.8 ± 1.5	0.6 ± 1.2

[a] Slope of linear regression ± 95% confidence interval on the slope.
[b] NA means no number assigned.

of toluene, benzene, o-xylene or m-xylene (Table 3). In a region of the water table aquifer that had been exposed to wood-creosoting wastes, the ATP content was much higher and the organisms could degrade the alkylbenzenes at an environmentally significant rate. This circumstance indicates that ATP contents can be used to identify regions in a polluted aquifer where natural renovation is occurring.

CONCLUSIONS

The capacity of subsurface material to degrade toluene was related to its ATP content. There was no detectable capacity of indigenous subsurface microorganisms to degrade toluene, other alkylbenzenes, or chlorobenzene whenever the ATP content of the subsurface material was at or below 0.05 $ng\,g^{-1}$. However, this observation is based on only two sites, and must be confirmed by further work before it can be considered to apply to the subsurface environment in general. When degradation was detected, it was rapid within the context of the slow flow of groundwater.

TABLE 3

Relationship between ATP content and biodegradation of alkylbenzenes in subsurface material from a site exposed to wood-creosoting wastes

Core No.	Position	ATP Content ($ng\,g^{-1}$)	Rate of disappearance % initial conc. per week			
			toluene	benzene	o-xylene	m-xylene
14D4	*Pristine material*	0.017				
14E4	Buff to brown coarse sand, 7.8 m deep	0.018				
7D + 7I + 7F	*Pristine material* Buff to brown coarse sand, 7.8 m deep		4.2 ± 2.1[a]	2.7 ± 1.3	3.1 ± 1.7	5.1 ± 3.8
7C1	Same as above — autoclaved		3.6 ± 2.5	4.4 ± 2.1	3.4 ± 1.1	3.2 ± 1.7
5C1	*Material exposed to wastes* Buff to brown coarse sand, 7.6 m deep and 2—4 m closer to the source of pollution than well No. 5B	0.37[b]				
6B	Same as above		18 ± 4.5	> 31	> 19	> 19
5B	Same as above		22 ± 5.3	> 31	> 21	> 22
5D	Same as above — autoclaved		3.4 ± 5.5	3.4 ± 5.5	3.0 ± 1.7	3.6 ± 4.9

[a] Slope of linear regression ± 95% confidence interval on the slope.
[b] Extraction performed in the laboratory.
Well 5B samples water from a plume of contamination that has been renovated by microbes in the aquifer.

The variability in ATP content and biodegradation potential was surprisingly high at the Lula, Oklahoma site. These properties varied over two orders of magnitude over a depth interval of only 4 m.

DISCLAIMER

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